

## LISTING OF CLAIMS

1-185. (Cancelled)

186. (New)

A method of producing a nonhuman mammal comprising the steps of:

- a. collecting sperm cells from a male of a nonhuman mammal species;
- b. establishing an artificial insemination sample having a low number of said sperm cells relative to a typical artificial insemination sample;
- c. freezing said artificial insemination sample having a low number of said sperm cells;
- d. thawing said artificial insemination sample having a low number of said sperm cells;
- e. inserting at least a portion of said artificial insemination sample having a low number of said sperm cells into a female of said nonhuman mammal species;
- f. fertilizing at least one egg within said female of said nonhuman mammal species at success levels statistically comparable to said typical artificial insemination sample; and
- g. producing an offspring nonhuman mammal.

187. (New)

A method of producing a nonhuman mammal as described in claim 186 wherein said step of collecting comprises the step of collecting sperm cells from a male of a nonhuman mammal species selected from the group consisting of bovines and equines.

188. (New)

A method of producing a nonhuman mammal as described in claim 186 or 187 wherein said steps of inserting and fertilizing are each accomplished in a field environment.

189. (New)

A method of producing a nonhuman mammal as described in claim 188 wherein said steps of inserting and fertilizing in a field environment comprise the steps of repetitively inserting a significant number of said artificial insemination samples into a significant number of said females of said nonhuman mammal species in rapid succession and in farm or ranch conditions.

190. (New)

A method of producing a nonhuman mammal as described in claim 186 wherein said female of said nonhuman mammal species has uterine horns and wherein said step of inserting comprises the step of inserting said artificial insemination sample both ipsi- and contra-lateral within said uterine horns of said female of said nonhuman mammal species.

191. (New)

A method of producing a nonhuman mammal as described in claim 186 or 187 wherein said female of said nonhuman mammal species has at least one uterine horn and wherein said step of inserting comprises the step of inserting said artificial insemination sample within said uterine horn.

192. (New)

A method of producing a nonhuman mammal as described in claim 191 wherein said step of inserting within said uterine horn further comprises the step of inserting said artificial insemination sample deep within said uterine horn.

193. (New)

A method of producing a nonhuman mammal as described in claim 191 wherein said step of inserting within said uterine horn further comprises the step of inserting said artificial insemination sample within said uterine horn through the use of embryo transfer equipment.

194. (New)

A method of producing a nonhuman mammal as described in claim 192 wherein said step of inserting deep within said uterine horn further comprises the step of inserting said artificial insemination sample deep within said uterine horn through the use of embryo transfer equipment.

195. (New)

A method of producing a nonhuman mammal as described in claim 186 wherein said step of inserting comprises the step of inserting said artificial insemination sample not later than about twelve hours after the time which is generally regarded as optimal for a single artificial insemination.

196. (New)

A method of producing a nonhuman mammal as described in claim 186 wherein said step of inserting comprises the step of inserting said artificial insemination sample not later than about seventeen hours from said step of establishing said artificial insemination sample.

197. (New)

A method of producing a nonhuman mammal as described in claim 186 wherein said step of inserting comprises the step of inserting said artificial insemination sample not later than about ten hours from said step of establishing said artificial insemination sample.

198. (New)

A method of producing a nonhuman mammal as described in claim 186 further comprising the steps of:

- a. determining the sex characteristic of a plurality of said sperm cells; and
- b. separating said sperm cells according to the determination of their sex characteristic.

199. (New)

A method of producing a nonhuman mammal as described in claim 198 wherein said steps of determining the sex characteristic of a plurality of said sperm cells and separating said sperm cells according to the determination of their sex characteristic comprise the steps of:

- a. providing a flow cytometer;
- b. establishing a cell source which supplies sperm cells to be sorted;
- c. chemically coordinating a sheath fluid to create a sheath fluid environment for said sperm cells which is coordinated with both a pre-sort and a post-sort sperm cell fluid environment;
- d. sensing a property of said sperm cells;
- e. discriminating between said sperm cells having a desired sex characteristic; and
- f. collecting said sperm cells having the desired sex characteristic.

200. (New)

A method of producing a nonhuman mammal as described in claim 198 wherein said steps of determining the sex characteristic of a plurality of said sperm cells and separating said sperm cells according to the determination of their sex characteristic comprise the steps of:

- a. providing a flow cytometer;
- b. establishing a cell source which supplies bovine sperm cells to be sorted;
- c. establishing a sheath fluid for said bovine sperm cells which contains about 2.9% sodium citrate;
- d. sensing a property of said bovine sperm cells;
- e. discriminating between said bovine sperm cells having a desired sex characteristic; and
- f. collecting said bovine sperm cells having the desired sex characteristic.

201. (New)

A method of producing a nonhuman mammal as described in claim 198 wherein said steps of determining the sex characteristic of a plurality of said sperm cells and separating said sperm cells according to the determination of their sex characteristic comprise the steps of:

- a. providing a flow cytometer;
- b. establishing a cell source which supplies equine sperm cells to be sorted;
- c. establishing a sheath fluid for said equine sperm cells which contains a hepes buffered medium;
- d. sensing a property of said equine sperm cells;
- e. discriminating between said equine sperm cells having a desired sex characteristic; and
- f. collecting said equine sperm cells having the desired sex characteristic.

202. (New)

A method of producing a nonhuman mammal as described in claim 198 wherein said steps of determining the sex characteristic of a plurality of said sperm cells and separating said sperm cells according to the determination of their sex characteristic comprise the steps of:

- a. providing a flow cytometer;
- b. establishing a cell source which supplies sperm cells to be sorted;
- c. establishing a sheath fluid for said sperm cells;
- d. sensing a property of said sperm cells;
- e. discriminating between said sperm cells having a desired sex characteristic; and
- f. collecting said sperm cells having the desired sex characteristic while cushioning said sperm cells from impact with a collector.

203. (New)

A method of producing a nonhuman mammal as described in claim 198 wherein said steps of determining the sex characteristic of a plurality of said sperm cells

and separating said sperm cells according to the determination of their sex characteristic comprise the steps of:

- a. providing a flow cytometer;
- b. establishing a cell source which supplies sperm cells to be sorted;
- c. establishing a sheath fluid for said sperm cells;
- d. sensing a property of said sperm cells;
- e. discriminating between said sperm cells having a desired sex characteristic; and
- f. collecting said sperm cells having the desired sex characteristic in a citrate collection fluid containing about six percent egg yolk.

204. (New)

A method of producing a nonhuman mammal as described in claim 199, 200, 201, 202 or 203 and further comprising the step of sorting said sperm cells at a rate of at least 1200 sorts per second.

205. (New)

A method of producing a nonhuman mammal as described in claim 186 and further comprising the step of using an ovulatory pharmaceutical to cause multiple eggs to be produced and wherein said step of fertilizing comprises the step of fertilizing a plurality of said eggs to produce multiple embryos, wherein said ovulatory pharmaceutical is injected in half day increments between any of days 2 and 18 of the estrus cycle.

206. (New)

A method of producing a nonhuman mammal as described in claim 205 wherein said step of using an ovulatory pharmaceutical to cause multiple eggs to be produced comprises the step of injecting a dosage of follicle stimulating hormone.

207. (New)

A method of producing a nonhuman mammal as described in claim 206 wherein said step of injecting said dosage of follicle stimulating hormone in approximately half day increments comprises a dosage level of 6, 6, 4, 4, 2, 2, 2, and 2 mg between days 9 and 12 inclusive of the estrus cycle and further comprising the step of injecting 25 and 12.5 mg of prostaglandin F-2-alpha on the sixth and seventh dosages, respectively, of said follicle stimulating hormone.

208. (New)

A method of producing a nonhuman mammal as described in claim 204 wherein said steps of determining the sex characteristic of a plurality of said sperm cells and separating said sperm cells according to the determination of their sex characteristic further comprise the steps of:

- a. staining said sperm cells with at least about 38 micro-molar content of stain; and
- b. concentrating said sorted sperm cells of said male nonhuman mammal.

209. (New)

A method of producing a nonhuman mammal as described in claim 187 further comprising the steps of:

- a. determining the sex characteristic of a plurality of said sperm cells; and
- b. separating said sperm cells according to the determination of their sex characteristic.

210. (New)

A method of producing a nonhuman mammal as described in claim 209 further comprising the step of chemically coordinating a sheath fluid environment for said sperm cells which is coordinated with both a pre-sort and a post-sort sperm cell fluid environment.

211. (New)

A method of producing a nonhuman mammal as described in claim 210 wherein said step of chemically coordinating a sheath fluid to create a sheath fluid environment for said cells which is coordinated with both a pre-sort and a post-sort sperm cell fluid environment comprises the step of establishing a cell source which supplies bovine sperm cells and the step of establishing a sheath fluid which contains about 2.9% sodium citrate.

212. (New)

A method of producing a nonhuman mammal as described in claim 210 wherein said step of chemically coordinating a sheath fluid to create a sheath fluid environment for said sperm cells which is coordinated with both a pre-sort and a post-sort sperm cell fluid environment comprises the step of establishing a cell source which supplies equine sperm cells and the step of establishing a sheath fluid which contains a hepes buffered medium.

213. (New)

A method of producing a nonhuman mammal as described in claim 209 wherein said step of separating said sperm cells according to the determination of their sex characteristic further comprises the step of cushioning said cells from impact with a collector.

214. (New)

A method of producing a nonhuman mammal as described in claim 213 wherein said step of cushioning said cells comprises the step of providing a collection container having a diameter of at least fifteen millimeters.

215. (New)

A method of producing a nonhuman mammal as described in claim 213 wherein said step of cushioning said cells comprises the step of avoiding impact of said cells with said collector.



216. (New)

A method of producing a nonhuman mammal as described in claim 213 wherein said step of cushioning said cells comprises the step of providing a collection container having stream matched physical characteristics.

217. (New)

A method of producing a nonhuman mammal as described in claim 186 wherein said step of establishing an artificial insemination sample having a low number of said sperm cells relative to a typical artificial insemination sample comprises the step of establishing an artificial insemination sample having less than about one-half the number of sperm cells relative to a typical artificial insemination sample.

218. (New)

A method of producing a nonhuman mammal as described in claim 186 wherein said step of establishing an artificial insemination sample having a low number of said sperm cells relative to a typical artificial insemination sample comprises the step of establishing an artificial insemination sample having less than about 10 percent the number of sperm cells relative to a typical artificial insemination sample.

219. (New)

A method of producing a nonhuman mammal as described in claim 186 wherein said step of establishing an artificial insemination sample having a low number of said sperm cells relative to a typical artificial insemination sample comprises the step of establishing an artificial insemination sample selected from the group consisting of: a bovine insemination sample of no more than one hundred thousand sperm cells, a bovine insemination sample of no more than two hundred fifty thousand sperm cells, a bovine insemination sample of no more than three hundred thousand sperm cells, a bovine insemination sample of no more than five hundred thousand sperm cells, a bovine insemination sample of no more than one

million sperm cells, and a bovine insemination sample of no more than five million sperm cells.

220. (New)

A method of producing a nonhuman mammal as described in claim 186 wherein said step of establishing an artificial insemination sample having a low number of said sperm cells relative to a typical artificial insemination sample comprises the step of establishing an artificial insemination sample selected from the group consisting of: an equine insemination sample of no more than one million sperm cells, an equine insemination sample of no more than five million sperm cells, an equine insemination sample of no more than ten million sperm cells, and an equine insemination sample of no more than twenty-five million sperm cells.